



Dissipation and transformation of 17 β -estradiol-17-sulfate in soil–water systems



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HIGHLIGHTS

- Soil organic carbon content significantly affects dissipation of 17 β -estradiol-17-sulfate in the soil–water systems.
- Hydroxylation is a major transformation pathway for 17 β -estradiol-17-sulfate.
- Deconjugation of 17 β -estradiol-17-sulfate occurs on the solid phase of the soil and to a lower extent compared to hydroxylation.

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ABSTRACT

In the environment, estrogen conjugates can be precursors to the endocrine-disrupting free estrogens, 17 β -estradiol (E2) and estrone (E1). Compared to other estrogen conjugates, 17 β -estradiol-17-sulfate (E2-17S) is detected at relatively high concentrations and frequencies in animal manure and surface runoff from fields receiving manure. To elucidate the lifecycle of manure-borne estrogens and their conjugates in the environment, the fate of radiolabelled E2-17S in agricultural soils was investigated using laboratory batch studies with soils of different organic carbon (OC) content (1.29% for topsoil versus 0.26% for subsoil). E2-17S was found relatively persistent in the aqueous phase throughout the duration of the 14 d experiment. The aqueous E2-17S persisted longer in the subsoil (half-lives (DT_{50}) = 64–173 h) than the topsoil (DT_{50} = 4.9–26 h), and the aqueous persistence of E2-17S depended on its initial concentration. The major transformation pathway was hydroxylation, yielding mono- and di-hydroxy-E2-17S (OH-E2-17S and diOH-E2-17S). Free estrogens, E2 and E1, were only observed in the sorbed phase of the soil at low concentrations (~1% of applied dose), which demonstrated that deconjugation and subsequent oxidation had occurred. Although deconjugation was not a major pathway, E2-17S could be a precursor of free estrogens in the environment.

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1. Introduction

Naturally occurring estrogenic hormones are endocrine-disrupting compounds (EDCs). For example, the lowest observable adverse effect level (LOAEL) of 17 β -estradiol (E2) is 10 ng L⁻¹ for aquatic organisms [1]. In US streams, E2 is frequently detected (frequency = 10.3%) at concentrations (9–160 ng L⁻¹) above the LOAEL [2]. However, E2 degrades within hours and is found immobile in laboratory soil experiments [3–5]. The discrepancies between field observations (high detection concentrations and frequencies) and

laboratory experiments (highly degradable and immobile), indicate that there are other mechanisms that facilitate transport and persistence of estrogens in the environment. Conjugated estrogens may contribute to the mobility and persistence of free estrogens in the environment. Estrogens are excreted in the urine primarily as conjugates of sulfate or glucuronide, which are more water-soluble than their counterpart free estrogens [6,7]. Furthermore, unlike free estrogens, conjugates are not biologically active [8], because they do not bind to estrogen receptors [9]. However, bacteria and enzymes can hydrolyze estrogen conjugates to yield the biologically active free estrogens, E2 or E1, in municipal and animal wastes [10,11]. Estrogen sulfate conjugates are more persistent, and are detected more frequently than glucuronides in municipal sewage systems [12], wastewater [13], and wastewater treatment plants (WWTPs) [14,15].

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Manure from animal feeding operations (AFOs) is land-applied as soil amendments, and can be a major source of steroid hormones and their conjugates to the environment. Livestock manure is estimated to contribute 90% of estrogens in the environment [16], and estrogen conjugates can comprise one-third of the total estrogen load from AFO manure [17]. Moreover, the highest conjugate levels measured in various AFO lagoons were sulfated forms, where estrone-3-sulfate (E1-3S), 17 β -estradiol-3-sulfate (E2-3S), 17 α -estradiol-3-sulfate (E2 α -3S), and 17 β -estradiol-17-sulfate (E2-17S) were measured at concentrations of 2–91 ng L $^{-1}$, 8–44 ng L $^{-1}$, 141–182 ng L $^{-1}$, and 72–84 ng L $^{-1}$, respectively [17]. When poultry (*Gallus gallus*) manure was applied to an agricultural field, no glucuronide conjugates were detected in surface runoff; only sulfate conjugates were found, and E2-17S concentration (107 ng L $^{-1}$) was higher than any other sulfate conjugates (E2-3S, E2 α -3S, and E1-3S) [18]. Additionally, runoff concentrations of E2-17S (0.3–3.9 ng L $^{-1}$) were higher than free E2 (0.5–1.9 ng L $^{-1}$) [19].

Free estrogens are non-volatile and relatively hydrophobic compounds, and they are sorbed rapidly by soils and sediments [4,5]. Degradation of free estrogens is reported to be rapid in soils, with half-lives of less than one day [20]. Considering that estrogen conjugates can act as precursors to free estrogens, it is imperative to understand the fate and transport of conjugates in the environment. Deconjugation potentials of estrogen sulfates have been studied in municipal sewage systems [12,21,22]; however, estrogen sulfates may behave differently in agricultural soils due to different microbial populations. The fate of E1-3S [23] and E2-3S [24] has been studied in pasture soils, and both sulfate conjugates were deconjugated to release free estrogens. A more recent study reported that a glucuronide conjugate, 17 β -estradiol-3-glucuronide (E2-3G), was quickly transformed into free E2 and E1 in soil–water slurries, which can be a significant contributor to free estrogens to the environment [25].

Compared to other conjugates, E2-17S is detected more frequently and at higher concentrations in AFO manure and in runoff from fields receiving manure. Swine manure is usually applied to agricultural land as slurry. A previous field study [26] by this research group reported that the manure application rates were 120 m 3 ha $^{-1}$, which supplied approximately 48 mm of water to the field. The surface runoff after manure slurry application can thus contain significant amounts of estrogen conjugates. To date, no studies have investigated the fate of E2-17S in soils, which is necessary to fully understand the behavior of manure-borne estrogens, the most significant source of steroid hormones to the environment. It is hypothesized in the present study that if E2-17S was applied to soils, then E2-17S could serve as a precursor to endocrine-disrupting, free estrogens in the environment. The objective of this study was to use batch experiments to determine the dissipation and transformation of E2-17S in the presence of agricultural soils.

2. Materials and methods

2.1. Analytical methods

All experiments were conducted using radiolabelled [^{14}C]E2-17S (specific activity = 241.8 Bq μg^{-1} ; radiochemical purity = 98%), which was synthesized in-house from [$4-^{14}\text{C}$]E2 (American Radiolabels, Inc., St. Louis, MO) [27]. Also, all radiometric methods were based on those developed by Shrestha et al. [28]. Briefly, for metabolite fractionation and quantification, a combination of high performance liquid chromatography (HPLC) and liquid scintillation counting (LSC) was used. The HPLC (Beckman Coulter Inc., Fullerton, CA) was equipped with a C18 column (250 mm \times 4.6 mm;

Table 1
Selected properties of soil samples.

	Topsoil	Subsoil
Depth (cm)	0–15	46–61
Organic matter (OM) (%)	1.70	0.50
Organic carbon (OC) (%)	1.29	0.26
Inorganic carbon (IC) (%)	0.00	0.00
pH	7.0	7.4
Cation exchange capacity (CEC) (cmol $_e$ kg $^{-1}$)	9.3	9.8
Sand:silt:clay (%)	83:10:7	90:4:6

Phenomenex; Torrance, CA), a System Gold 508 auto-sampler, a 126 solvent module pump, a 168 UV detector, and a Gilson FC 204 fraction collector (Middleton, WI). The HPLC solvents and gradient were identical to the previous study [28]. Aliquots of fraction-collected samples (1 mL) from the HPLC were transferred to 6 mL scintillation vials, to which 4 mL of Ecolite scintillation cocktail (MP Biomedicals, Santa Ana, CA) were added, and then assayed for radioactivity for 10 min using LSC (1900 CA, Packard, Downers Grove, IL).

Metabolites were identified and characterized by liquid chromatography with tandem mass spectroscopy (LC-MS/MS) in negative-ion mode (ESI-). The mass spectrometer used for the analysis was an Ultima API-US Quadrupole-Time of Flight mass spectrometer (Waters, Beverly, MA) equipped with an electrospray ionization source. The capillary voltage was 2.33 kV, cone voltage was 55 V, and source and desolvation temperatures were 120 and 400 °C, respectively. The cone and desolvation gas flows were 0 and 500 L h $^{-1}$, respectively. The HPLC column was a Symmetry C18, 3.5 μm , 2.1 mm \times 100 mm with a 2.1 mm \times 10 mm guard column (Waters, Milford, MA). The initial mobile phase consisted of 60% 95:5 water:acetonitrile (solvent A) and 40% acetonitrile (solvent B). A linear gradient to 100% B was used from 0 to 10 min followed by a hold for 5 min at a flow rate of 0.2 mL min $^{-1}$.

To determine non-extractable, or irreversibly bound, radioactive residue in soil, combustion analysis was used. A mass of 0.1 g of extracted, air-dried soil (5 \times) was placed in fiber thimbles and combusted in a Packard Model 307 Oxidizer (Downers Grove, IL). Radiolabelled carbon dioxide was trapped with 8 mL of CarboSorb E (Waltham, MA) and analyzed by LSC after mixing with 12 mL of Permafluor cocktail (Waltham, MA).

2.2. Batch experiments

The soils used for the batch experiments were sampled from southeastern North Dakota, which is from the Hecla-Hamar Series, a loamy fine sand (sandy, mixed, frigid Oxyaquic Hapludolls). Previous laboratory [25,29–32] and field [26,33,34] studies from this research group used the same soil type, which aided in interpreting observations and discerning various fate and transport processes of steroid hormones in the environment. Soil samples were collected from two depths: topsoil (0–15 cm) (A horizon) and subsoil (46–61 cm) (C horizon). Swine manure slurry is applied to agricultural fields either by direct surface application or by injection beneath the upper 15 cm of soil [26]. Consequently, the two soil depths can be potentially affected by manure slurry. The properties (Table 1) of the two soils were similar except for their organic carbon (OC) content. Before conducting the batch experiments, the soil samples were air-dried and passed through a 2 mm sieve.

All batch experiments were conducted at room temperature (23 ± 1 °C), and followed methods from a previous study [25]. [^{14}C]E2-17S (in 10 μL MeOH) was added at four different concentrations: 0.6, 2.9, 8.9, and 30 mg L $^{-1}$, to triplicate 10 mL glass vials containing a soil–water mixture (1.6 g and 8 mL of 0.01 M CaCl $_2$ solution, respectively). Controls consisted of 0.01 M CaCl $_2$ and 0.6 mg L $^{-1}$ of [^{14}C]E2-17S with no soil. The concentration ranges in this study were higher than concentrations normally found

in the environment, which were used to ensure the adequate quantification and high resolution for the parent compound and potential metabolites by LSC. Similar concentration ranges have been selected for a radiolabelled glucuronide estrogen, [¹⁴C]E2-3G [25]. The batch vials were agitated by rotation from top to bottom (360° every 5 s) for 14 d (336 h). Aliquots of 100 and 150 µL were removed from the aqueous phase using a sterile syringe at 4, 8, 24, 48, 72, 168, and 336 h for LSC and HPLC analysis. The HPLC aliquot was passed through a 0.45 µm pore-size glass fiber filter, stored in a LC-MS/MS glass vial with formaldehyde (2.7% of final volume), and frozen until further analysis.

At each sample time, a single batch vial (hereafter called "stop vials") was removed from the low dose group (0.6 mg L⁻¹) and analyzed for parent compound and metabolites that partitioned to the sorbed phase. The removed "stop vials" were preserved with formaldehyde (2.7% of final volume) and then centrifuged. After centrifuging, the supernatant was transferred into new, clean batch vials, and both the liquid and soil samples were frozen until further analysis. To determine the radioactive residue bound to the soil, the soil was first extracted by water (5 mL × 3) and then by acetone (5 mL × 3) during 30 min of sonication. Aliquots (500 µL) from the water and acetone extracts were assayed for total radioactivity by LSC. The water extracts were then dried with a centrifugal rotary evaporator (Savant, Farmingdale, NY), and the acetone extracts were dried under a gentle stream of nitrogen. The extracts were reconstituted in methanol for HPLC analysis. Radioactivity that was extractable from the soil was considered reversibly sorbed; and non-extractable radioactivity was considered irreversibly sorbed, which was analyzed by soil combustion as described in Section 2.1. Additionally, no gas phase analysis of the batch vial headspace was conducted because earlier studies showed that mineralization of free [30] and glucuronide conjugated estrogen [25] did not occur under these soil conditions.

2.3. First-order kinetics

Dissipation of [¹⁴C]E2-17S in the aqueous phase was described using the following first-order kinetic model (SigmaPlot® 2000 for Windows®; version 6.00 SPSS Inc.):

$$\text{Parent compound : } \frac{C}{C_0} = e^{-kt} \quad (1)$$

where C/C_0 is the relative concentration of [¹⁴C]E2-17S, t (h) is time, and k (h⁻¹) is the dissipation rate constant of [¹⁴C]E2-17S. Dissipation times for 50% (DT_{50}) and 90% (DT_{90}) of the parent compound were directly calculated using the first-order dissipation rate constants ($DT_{50} = \ln 2/k$ and $DT_{90} = \ln 10/k$).

2.4. Statistical analysis

Significance of main effects and interactions was determined using analysis of variance (ANOVA). Tukey's and Student's *t*-tests were used to determine whether there were significant differences between levels. For all statistical analysis, an α of 0.05 was used, and a probability of $p \leq 0.05$ was considered significant. The program JMP (version 9.0.2 SAS Institute Inc.) was used for all statistical analysis.

3. Results and discussion

3.1. Aqueous phase observations

3.1.1. Parent compound dissipation

For the topsoil (Fig. 1A), aqueous concentrations of E2-17S reached steady-state between 48 h and 72 h for all initial

Table 2

Parameter estimates with standard deviation for [¹⁴C]17β-estradiol-17-sulfate using the first-order kinetic model under multiple initial concentrations.

Initial concentration (mg L ⁻¹)	k (h ⁻¹)	r^2	DT_{50} (h)	DT_{90} (h)
<i>Topsoil</i>				
30	0.026 (0.003)	0.980	26	88
8.9	0.030 (0.005)	0.937	23	77
2.9	0.033 (0.005)	0.955	21	71
0.6	0.143 (0.022)	0.967	4.9	16
<i>Subsoil</i>				
30	0.004 (0.001)	0.869	173	576
8.9	0.006 (0.000)	0.989	108	360
2.9	0.007 (0.001)	0.991	99	329
0.6	0.011 (0.003)	0.827	64	213

concentrations. The aqueous E2-17S concentrations in the subsoil never reached steady-state, but continued to dissipate for the 14 d duration of the experiment (Fig. 1B). The first-order dissipation rate constants (k) of the topsoil were greater than the subsoil values (Eq. (1); Table 2), reflecting faster aqueous dissipation of E2-17S, which can be attributed to greater sorption capacity and faster transformation. Soil with higher OC would result in higher sorption capacity of estrogens [4,5]. Also, higher soil OC and microbial biomass would result in greater biotic metabolism of sulfate conjugates [23,24]. Although the microbial activity was not measured in the present soils, it is widely reported that microbial biomass and enzymatic activities decrease with increasing soil depth [35]. Similar differences in aqueous dissipation between the subsoil and topsoil were also observed for E2-3G [25]. However, compared to E2-3G, E2-17S persisted longer in the aqueous phase, where the DT_{50} values for E2-17S (Table 2) were greater than values reported for E2-3G in the topsoil ($DT_{50} = 1.5\text{--}3.3$ h) and subsoil ($DT_{50} = 41\text{--}116$ h) [25]. Moreover, the DT_{50} values for E2-17S were greater than values reported for the free E2 (0.96–12 h) in various soils [20,36], indicating the greater persistence of E2-17S.

Aqueous dissipation of E2-17S was greater for lower initial concentrations (Fig. 1A and B). Similar concentration-dependent dissipation in soils was observed for 17β-trenbolone acetate [37] and for E2-3G [25], which is attributed to enzymatic saturability. In activated sludge, however, Chen and Hu [38] reported that dissipation rates increased with increasing concentrations of E2, E2-3G, and E2-3S, which is attributed to the induction of greater microbial activities by the added substrate. These contrasting observations between the soil studies (current study, [25,37]) and activated sludge study [38] may be caused by the greater biological activity, diversity, and capacity of the activated sludge compared to the soils.

3.1.2. Metabolite formation

Metabolites with higher polarity than E2-17S were detected in the aqueous phase, and were characterized as mono-hydroxy-E2-17S (OH-E2-17S) and di-hydroxy-E2-17S (diOH-E2-17S). The LC-MS/MS spectrum was consistent with the formation of OH-E2-17S ([M-H]⁻ ion at *m/z* of 367.12 and fragments of 349.13 and 96.96) and diOH-E2-17S ([M-H]⁻ ion at the *m/z* of 383.13 and fragments of 365.10 and 96.96); however, without standards, the position of the hydroxyl groups could not be determined definitively. The hydroxylated metabolites were already present at the first sample time of 4 h (Fig. 1C–F), indicating that hydroxylation was a very rapid process.

The formation of hydroxylated E2-17S metabolites was likely caused by oxidation by soil enzymes (e.g. oxidases/hydroxylases). *In vitro* studies have demonstrated enzymatic processes that govern hydroxylation of E2, E2-17S, and other aromatic compounds. For example, hydroxylation of E2 and E2-17S at the C-2- or C-4-positions is catalyzed by microsomal cytochrome P450 enzymes

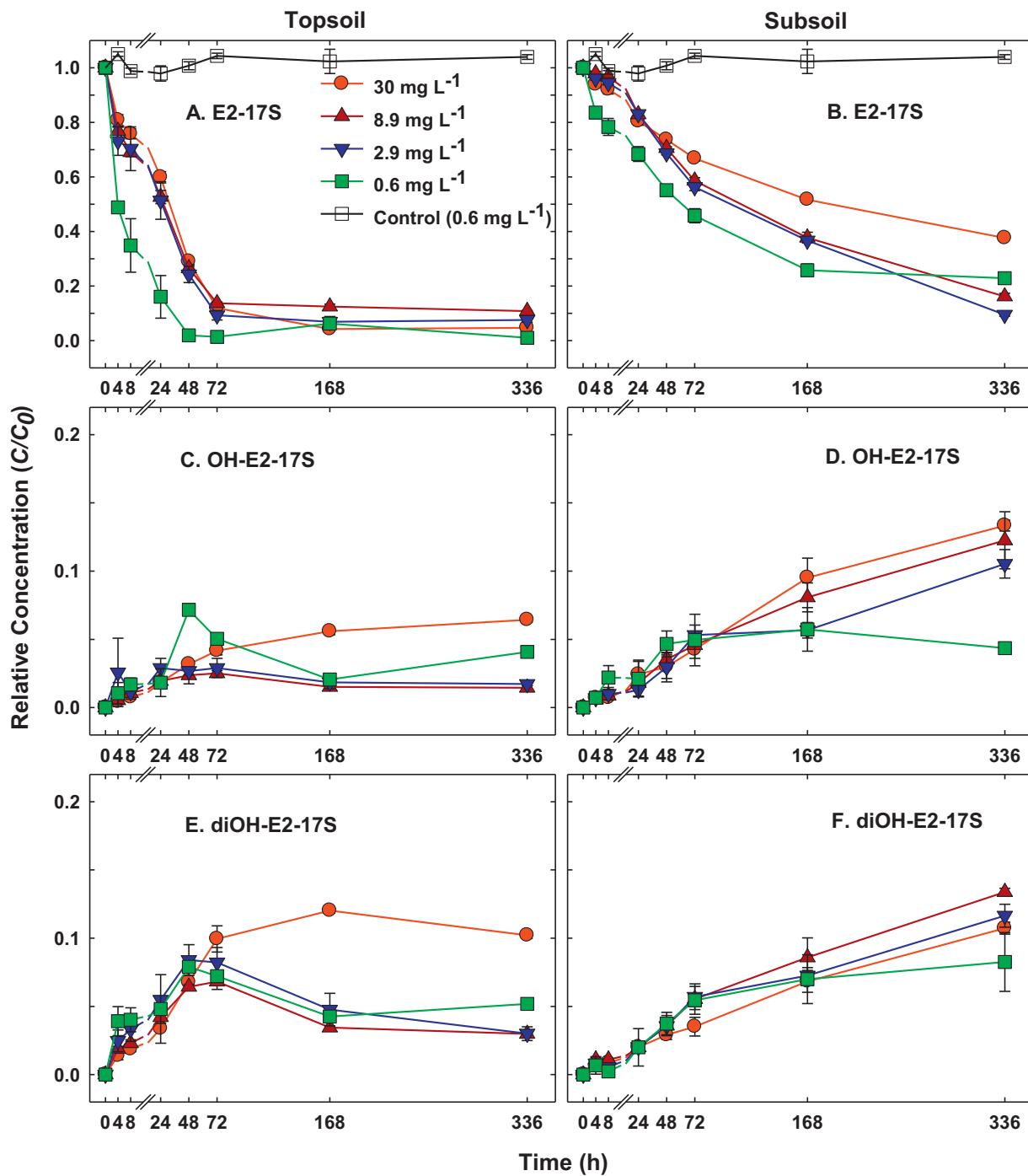


Fig. 1. Aqueous concentration of $[^{14}\text{C}]17\beta\text{-estradiol-17-sulfate}$ and its metabolites in topsoil and subsoil through time under different initial concentrations. The relative concentration represents the ratio between the measured concentration and the initial applied concentration of $[^{14}\text{C}]17\beta\text{-estradiol-17-sulfate}$ in the present and other figures. Dotted symbols represent the average of three replicates of the measured data. Error bars represent one standard deviation.

harvested from rat (*Rattus norvegicus*) liver [39]. Also, during incubation with human placental microsomes in an NADPH-generating system, E2-17S is hydroxylated to 2- and 4-OH-E2-17S [40]. Additionally, hydroxylation of E2-17S occurs when incubated with microsomes from female rat liver [41]. Outside of these *in vitro* studies, the present study appears to be unique in demonstrating hydroxylation of conjugated or free estrogens in soils. Soil microorganisms are reported to oxidize aromatic compounds to hydroxylated intermediates with mono- or di-oxygenases, which is then followed by ring-cleavage [42].

3.2. Sorbed phase observations

The total radioactive residue in the reversibly sorbed phase was relatively low for both soils (~15% of applied dose; Fig. 2). At the final sample time (336 h), the total radioactive residue in the irreversibly sorbed phase was greater than the reversibly sorbed phase for both the topsoil (irreversible ^{14}C = 70% of applied dose) and subsoil (irreversible ^{14}C = 45% of applied dose) (Fig. 2). Using the same soil type as the present study, Fan et al. [30] found that 73% of the applied radiolabelled E2 was irreversibly bound to soil

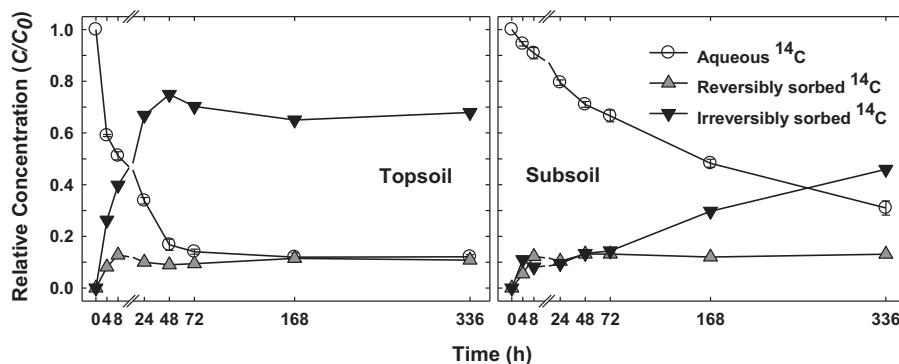


Fig. 2. Distribution of radioactivity in the aqueous, reversibly sorbed, and irreversibly sorbed phase of topsoil and subsoil through time. The initial [^{14}C]17 β -estradiol-17-sulfate concentration was 0.6 mg L^{-1} . Standard deviation error bars were available only for the aqueous data ($n=3$), while the reversibly and irreversibly sorbed data were based on the "stop vials" ($n=1$).

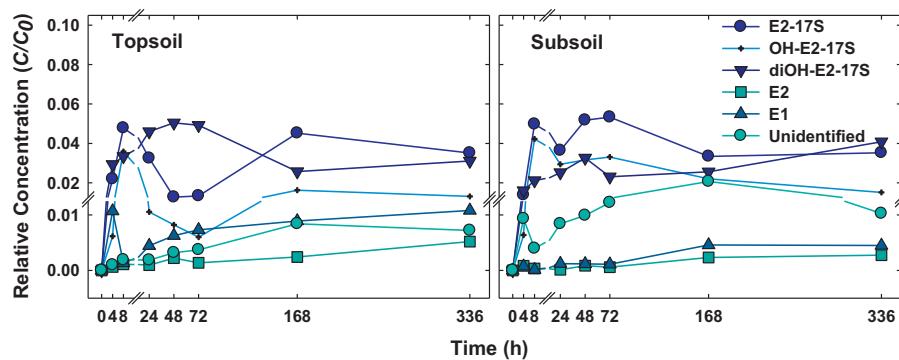


Fig. 3. Reversibly sorbed concentration of [^{14}C]17 β -estradiol-17-sulfate and its metabolites in topsoil and subsoil through time. Data were obtained from the "stop vials" with an initial [^{14}C]17 β -estradiol-17-sulfate concentration of 0.6 mg L^{-1} ($n=1$).

(non-extractable fraction). The pH, CEC, and texture (Table 1) of the two soils were similar, which indicated that the difference of radioactive residue levels in the sorbed phase likely resulted from the different OC content. Sorption of estrogens to soils and sediments is considered a hydrophobic interaction, with organic matter as the major sorption domain [5]. Although the metabolites could not be characterized in the irreversible phase, irreversible sorption is due to interactions between the phenolic group of estrogenic compounds and humic acids and/or mineral surfaces to form hydrogen and covalent bonds [43].

Compounds detected in the reversibly sorbed phase (extractable fraction) were diOH-E2-17S, OH-E2-17S, E2-17S, E2, estrone (E1), and an unknown metabolite (Fig. 3), in order of highest to lowest polarities based on the HPLC elution times. The most significant observation was the presence of free E2 and E1 in the sorbed phase, which were not detected in the aqueous phase. Although the measured concentrations of E2 and E1 were relatively low (~1% of applied dose; Fig. 3), these results demonstrated that E2-17S could be hydrolyzed to form free estrogens in agricultural soils. Additionally, an unknown metabolite that was more hydrophobic than E1 was consistently observed in the reversibly sorbed phase for both soils; however, it could not be characterized due to the low levels of recovery. This unidentified hydrophobic compound was present at lower concentrations in the topsoil compared to the subsoil throughout the entire experiment (0.7% vs. 1% of applied dose at 336 h; Fig. 3).

The observed E2 and E1 on the sorbed phase demonstrated that E2-17S was first hydrolyzed to form E2, which was subsequently oxidized to yield E1. Deconjugation of conjugate moieties is considered irreversible, and enzymes (i.e. sulfatases) are required to hydrolyze sulfate conjugates [11]. D'Ascenzo et al. [12] found that

an acclimation period of 10 h was required to deconjugate E2-3S in domestic wastewater due to low inherent arylsulfatase activity. Also, arylsulfatase enzymes were found to be responsible for deconjugation of E2-3S in natural soils [24]. Arylsulfatases can distribute in the solid or aqueous phase of soils, which permits E1-3S deconjugation in both compartments [23]. In the present study, the deconjugated free estrogens, E1 and E2, were only observed on the sorbed phase, suggesting that sulfatase enzymes were only active on the solid phase. The oxidation of E2 into E1 was rapid, where E1 was detected without a lag period, occurring at the first sample time (4 h) (Fig. 3). As reported, oxidation of E2 is a fast process in soil with a half-life of 12 h [20], and can occur on Mn-oxide reaction sites of soil surface [44]. Because deconjugation of E2-17S appeared to be a sorbed-phase process, the proximity to surface Mn-oxide reaction sites may explain the observed immediate oxidation of E2 into E1. Additionally, the predominance of E1 compared to E2 (Fig. 3), was consistent with the observation that E1 is more stable than other naturally occurring estrogens in the environment [17].

3.3. Deconjugation/hydrolysis

The present study observed that hydroxylation of E2-17S occurred approximately 10 times greater than deconjugation/hydrolysis. Similarly, deconjugation of other estrogen sulfates only occurred to a limited extent in sewage treatment systems and soils. For instance, an 8 h activated sludge and crude sewage batch study showed that aqueous concentrations of E1-3S and ethinylestradiol-3-sulfate remained between 87% and 94% of the applied dose, while free estrogens were only 3–7% of the applied dose [21]. Additionally, deconjugation of E1-3S and E2-3S was negligible in batch experiments with raw sewage and river water [22].

In a 10 d soil incubation study (sandy loam; OC = 1.1%; 25 °C), Scherr et al. [24] found that the primary metabolite of E2-3S was E1-3S (55–68% of applied dose), indicating that oxidation was the predominant process. Deconjugation of E2-3S to yield E2 also occurred, but to a much lower extent (2.7–3.5% of applied dose) compared to oxidation. Despite the different soil types and experimental designs, Scherr et al. [24] and the current study both showed that deconjugation/hydrolysis was not the dominant metabolism pathway for E2-3S or E2-17S in agricultural soils.

In contrast to sulfate conjugates, the primary transformation pathway for the glucuronide conjugate, E2-3G, in the similar soil–water systems was deconjugation, which produced maximum aqueous concentrations of E2 (18% of applied dose) within 24 h [25]. The different metabolism pathways of E2-17S and E2-3G in soils are consistent with observations in sewage systems that glucuronide conjugates were more susceptible to deconjugation than sulfate conjugates [12,21]. In addition to difference in conjugate moieties (sulfate vs. glucuronide), the site of conjugation may also influence the metabolism pathways. Glucuronide conjugates with the moiety located on the D-ring are more resistant to hydrolysis than A-ring glucuronides [21]. The effect of conjugation sites would also explain the more rapid degradation of E2-3S (half-life of 1.5 h) compared to no degradation of 17β-estradiol-3,17-sulfate in batch studies of WWTP activated sludge [45]. The recalcitrance of E2-17S to deconjugation in the present study can result from the relatively stable sulfate moiety compared to glucuronide, as well as the conjugation site, i.e. C-17.

4. Conclusions

The objective of this study was to investigate the fate of E2-17S in natural agricultural soils, under the context of understanding the fate and transport of manure-borne estrogens. The results showed that soil OC content significantly influenced the aqueous dissipation of E2-17S. Additionally, hydroxylation was found to be the primary transformation pathway of E2-17S. Deconjugation/hydrolysis of E2-17S did occur, but it was a minor transformation pathway compared to hydroxylation, with only low concentrations of free estrogens (i.e. E2 and E1) being released on the sorbed phase. Although, E2-17S was more persistent than the glucuronide conjugate (E2-3G), it has a lower potential of releasing free estrogens to the environment. Nonetheless, due to the large amounts of manure-borne estrogen conjugates arising from AFOs, even 1% of free estrogens deconjugated from E2-17S may result in environmental levels higher than the LOAEL. Therefore, the relatively stable estrogen conjugate, E2-17S, cannot be excluded as a precursor of free estrogens in the environment.

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